Exploring the homogeneity of terrestrial subterranean communities at a local spatial scale

Exploring the homogeneity of terrestrial subterranean communities at a local spatial scale

Stefano Mammola[1,*], Nicolò Chiappetta[2], Pier Mauro Giachino[3], Dragan Ž. Antić [4], Marzio Zapparoli[5], Marco Isaia[2,**]

1. Molecular Ecology Group (MEG), Water Research Institute, National Research Council of Italy (CNR-IRSA), Verbania Pallanza, Italy

2. Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy

3. Word Biodiversity Association Onlus, Verona, Italy

4. University of Belgrade – Faculty of Biology, Institute of Zoology, Belgrade, Serbia

5. Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Tuscia University, Viterbo, Italy

* corresponding author: stefano.mammola@cnr.it

** corresponding author: marco.isaia@unito.it

Author contribution

SM and MI conceived the study. SM, NC, and MI performed fieldwork. NC sorted specimens. MI identified spiders and harvestmen. DŽA identified millipedes. MZ identified centipedes. PMG identified coleopterans and other insect orders. MI and NC performed GIS analyses. SM performed statistical analysis and wrote the first draft of the paper. All authors contributed to the writing and approved the final submission.

Running title

Turnover in subterranean communities

Statements

Authors disclose any actual or potential conflict of interest.

ABSTRACT

1. While caves are generally perceived as isolated habitats, at the local scale they are often interconnected via a network of fissures in the bedrock. Accordingly, caves in close proximity are expected to host the same, or very similar, animal communities.

2. We explored the extent to which subterranean arthropod communities are homogenous at a local spatial scale of less than 1 km^2, and which cave-specific environmental features result in a departure from the expected homogeneous pattern. We approached this question by studying richness and turnover in terrestrial arthropod communities of 27 caves in a small karst massif in the Western Italian Alps.

3. Obligate subterranean species were homogeneously distributed among caves and were not influenced by seasonality. The only factor driving their presence and abundance was the distance from the cave entrance, with deeper caves yielding a greater diversity and abundance of species.

4. We observed significant spatial and temporal turnover in non-obligate subterranean species. In summer, there was a significant homogenization of the community and a more even distribution of non-obligate species among sites; in winter, these species were missing or found exclusively at greater depths, where environmental conditions were more stable. Furthermore, caves at lower elevations yielded, on average, a greater diversity and abundance of non-obligate species.

5. We demonstrated that the theoretical expectation of no turnover in community composition in caves in close proximity is not always met. Given that turnover can be mostly attributed to seasonal patterns and sampling depth, our findings have implications for planning sampling and monitoring activities in caves.

Keywords: Beta diversity; Community ecology; Pitfall traps; Spatial turnover; Subterranean biology; Temporal turnover

INTRODUCTION

Ecologists and evolutionary biologists have frequently compared caves to islands (Culver, 1970; Snowman *et al.*, 2010; Esposito *et al.*, 2015; Fattorini *et al.*, 2016; Itescu, 2019; Mammola, 2019). In fact, from the perspective of a specialized subterranean organism, a cave is a patch of suitable habitat within a matrix of unsuitable habitats. It is understood that island-like properties of caves emerge mostly at broad geographical scales (Stoch & Galassi, 2010), wherever the extent of the study area is large enough to encompass geological discontinuities or other natural barriers limiting subterranean connectivity (see Fig. 2 in Mammola, 2019). For example, studies exploring the extent to which subterranean communities vary in their composition at continental scales have documented higher rates of turnover (β-diversity) than those typically observed in surface habitats (Zagmajster *et al.*, 2014; Mammola *et al.*, 2019). Yet, it is understood that β-diversity patterns are strongly scale dependent (Jarzyna & Jetz, 2018), meaning that the "caves as islands" view rarely holds true at the local scale (Mammola, 2019).

According to a modern understanding of the subterranean realm, a cave is nothing but a human-accessible void in the ground. Subterranean organisms not only inhabit caves, but can thrive in a large breadth of lightless habitats including networks of fissures in the bedrock and shallow habitat pores not directly accessible to humans (Culver & Pipan, 2010; Giachino & Vailati, 2010; Mammola *et al.*, 2016; Pipan & Culver, 2017; Ficetola *et al.*, 2019). Indeed, we are starting to perceive caves as small "windows" opening to a vaster world, the subterranean one, allowing us to take a glimpse of what happens below our feet (Howarth, 1983; Uéno, 1987). If microcavernous habitats ensure subterranean habitat connectivity at the local scale, low values of β-diversity should be observed when studying biological communities of caves in close proximity. This pattern was recently confirmed by research exploring the spatial distribution of invertebrate species over the cross-section of the Trnovski Gozd massif in Slovenia, demonstrating a limited distinction between shallow (0–50 m) and deep (up to 800 m) cave communities within this karst massif (Trontelj *et al.*, 2019).

The expectation of no community turnover among caves at restricted spatial scales is not, however, always met. Even when sampling caves in close proximity that theoretically should harbour the same fauna, slight or even conspicuous species turnover is observed. These may be the result of daily and seasonal changes in community composition (Lunghi *et al.*, 2017; Mammola *et al.*, 2017), of local habitat unsuitability due to a combination of specific environmental conditions (Jiménez-Valverde *et al.*, 2017), of competition dynamics that may lead to the exclusion of certain species from a given community (Mammola & Isaia, 2014), or even simply the consequence of imperfect detection (Ficetola *et al.*, 2018) when sampling such structurally-complex environments (Wynne *et al.*, 2018,

2019).

Here, we seek to understand the extent to which habitat connectivity determines a homogenization of subterranean biological communities at local scales, and which cave-specific environmental conditions may determine a departure from this expected pattern. We used a geographically restricted area, the cave system of Comba dell'Infernotto (Chesta & Elia, 2004), as a uniquely suited study site for this purpose, in that it hosts a great diversity of caves in an area of approximately 1 km^2. These caves encompass a wide range of environmental conditions and host a rich and diversified fauna, with several specialized subterranean species. Therefore, this system is well-suited for exploring the ecological determinants of α- and β-diversity patterns at the local scale.

Our null hypothesis was that community composition is the same in all caves within the considered subterranean system. In other words, we expected to observe comparable levels of α-diversity in all caves, and β-diversity values close to zero in pairwise comparisons among caves. We hypothesize that departures from such theoretical expectations should be linked to seasonal patterns in community composition and/or due to a combination of unsuitable local environmental conditions.

MATERIAL AND METHODS

Study area

The study was carried out in the cave system of Comba dell'Infernotto (or Infernetto) (7.4° E, 44.2° N), in the municipality of Valdieri, Maritime Alps, Province of Cuneo, Piedmont, Italy (Figure 1). With more than 40 caves and mines currently documented, this small karst area yields the greatest number of subterranean sites in the province of Cuneo (Chesta & Elia, 2004). This area is included within the boundaries of the Site of Community Importance IT1160056 "Alpi Marittime" (European Habitat Directive 43/92).

For this study, we selected 27 subterranean sites (Fig. 1) within the area of Comba dell'Infernotto, with a maximum distance of 1 km between sites. Other caves in this system were not investigated due to access difficulties. We sampled both caves and old abandoned mines in the same area, ranging from 4 to 861 m in length, from 0.5 to 35 m^2 in the size of the cave entrance, and from 1000 to 1200 m in the elevation of cave entrances (Table 1). All sites entrances were in a dense beech forest (*Fagus sylvatica* L.).

Sampling design

We sampled invertebrates in caves using pitfall traps (diameter 9 cm, volume 40 ml). To minimize impacts on invertebrate populations, only one pitfall trap per site was used. We installed traps at the

ground level away from accumulation of organic material. We covered each trap with a flat stone to shelter it from percolating water (Růžička, 1982). We filled traps with brine (supersaturated preserving solution of water and NaCl; Giachino & Vailati, 2010) and baited them with cheese. We acknowledge that the specific use of a bait, by exerting different attraction on different species, may bias the composition of the sampled arthropod assemblages. Nevertheless, this represented a homogeneous bias across the sampling, thus did not compromise our specific aims.

To evaluate seasonal patterns in community composition, we trapped invertebrates once in summer, from June to September 2017, and once in winter, from November 2017 to April 2018. We were forced to overextend the winter sampling period (6 vs 4 months in summer) due to a late snow melt.

We measured the linear distance from the cave entrance using an extendable tape. Moreover, we estimated the subjacency of each trap (i.e., the vertical distance from the surface; Mammola et al., 2017) using GIS, marking the position of the trap on available geological surveys of Chesta and Elia (2004) and plotting the latter in plan view on a georeferenced topographical map. We derived subjacency for traps in caves below 10 m in length by direct measurements. We derived total cave length and maximal subjacency of each cave from Chesta and Elia (2004), or measured these parameters directly in the field whenever this information was not available in speleological literature. We approximated the area of each cave entrance by multiplying its base by its height. For each cave, we also recorded by eye the presence of percolating water in the two seasons.

We measured temperature at each pitfall trap with EL-USB-2+ dataloggers (Lascar Electronics, Salisbury, United Kingdom), programmed to record a temperature measure (accuracy of ± 0.5°C) every six hours (0:00, 6:00, 12:00, 18.00) throughout the two sampling periods.

Species identification and ecological classification

Specimens were identified to the lowest possible taxonomic rank by experts of the different groups (see author contribution for details). For the purpose of the analyses, we assigned each species to one of the two major ecological groups commonly used to explore subterranean diversity patterns (e.g., Novak et al., 2012; Niemiller & Zigler, 2013; Trontelj et al., 2019): obligate subterranean species ('troglobionts'; Sket, 2008; Trajano & de Carvalho, 2017), and non-obligates ('non-troglobionts'; Novak et al., 2012) (Supplementary material Appendix S1). We based this classification mainly on the expert opinion of different taxonomists involved in this work, but also using data and morphological traits associated with subterranean life (loss of eyes and body pigment) from the literature.

Statistical analyses

Data supporting the study are deposited in figshare (doi: 10.6084/m9.figshare.12005934 Note that the hyperlink will become available upon acceptance). All analysis were conducted in R (R Core Team, 2018). We used Poisson generalized linear models (GLMs) to explore factors driving alpha diversity (α) and the abundance of obligate and non-obligate subterranean species in our study sites. We followed the general protocol by Zuur & Ieno (2016) for conducting regression-type analyses, whereby we:

i) performed data exploration on the initial dataset, aiming to verify the presence of outliers, remove collinear predictors, and detect potential interactions among predictors (Zuur *et al.*, 2009);

ii) constructed Poisson GLMs to test for significant relationships between the dependent variables and our explanatory predictors. We included potential interactions among predictors only if these were detected during data exploration (see point i);

iii) performed a step-wise model selection on each model with the '*step1*' R function, in order to obtain a Minimum Adequate Model (MAM) for each of the dependent variables, minimizing the Aikaike Information Criterion (AIC) value; and

iv) validated each model with the aid of the R package '*performance*' (Lüdecke *et al.*, 2020). In this phase, we tested each model for over-dispersion, and switched to a negative binomial distribution if models were over-dispersed (Gelman & Hill, 2007).

To investigate spatial and temporal turnover in community composition, we estimated pairwise β-diversity among caves and between the two seasons using the framework proposed by Carvalho et al. (2012) and Cardoso et al. (2014), whereby β is expressed as:

$$\beta_{total} = \beta_{replacement} + \beta_{richness}$$

$\beta_{richness}$ is the turnover in community composition explained by species loss/gain alone, and $\beta_{replacement}$ is turnover in community composition explained by replacement of species alone. Thus, this framework allowed us to distinguish between these two processes (loss/gain vs replacement) underlying changes in community composition. β-diversity was calculated with the function '*beta*' in the R package '*BAT*' (Cardoso *et al.*, 2015, 2020), using species abundances as input data. We graphically explored changes in β-diversity values among caves in the two sampling seasons using density plots. We used a Wilcoxon rank sum test with continuity correction to test if median $\beta_{replacement}$ and $\beta_{richness}$ values were significantly different between the two seasons for both obligate and non-obligate subterranean species.

RESULTS

α-diversity and abundance of species

In total, we trapped 3,026 specimens belonging to 11 arthropod orders, 12 molluscans, and 1 nematode (Supplementary material Appendix S1). Dipterans, both adults and larvae, were the most abundant organisms in our traps (1,673 specimens), followed by coleopterans (616), diplopods (308), and acari (208). The pitfall trap placed in cave 11 (a small cave near Barôn Litrôn) was lost in summer, and therefore this site was excluded from the analysis.

Due to malfunctioning, half of the dataloggers did not measured temperature correctly throughout the sampling period Since the removal of these missing data would have halved the sample size of the dataset, and considering that cave temperature was significantly associated with sampling season, we did not include any temperature-derived variable in the regression analysis. Furthermore, we found that the categorical variable "presence of percolating water" was significantly associated with cave length. Cave length, cave maximum subjacency, and pitfall trap subjacency were also collinear with the distance of the pitfall from the entrance (all Pearson $r > 0.7$). Therefore, we included only the distance of the pitfall from the entrance, cave elevation, cave entrance size, and sampling season in the initial regression models We successfully fitted and validated models for all four dependent variables. Poisson α-diversity models were not over-dispersed [Obligates: Dispersion Ratio (DR)= 0.78; Pearson's χ^2= 36.57; p= 0.86; Non-obligates: DR= 1.21; χ^2= 55.56; p= 0.16]. Conversely, abundance Poisson GLMs were significantly over-dispersed (Obligates: DR= 12.04; χ^2= 565.73; p< 0.001; Non-obligates: DR= 35.74; χ^2= 1643.84; p< 0.001) and thus, we opted for a negative binomial distribution to model abundance. Results of model selection are presented in Table 2, while estimated regression coefficients and p-values are given in Table 3. α-diversity (Figure 2a) and abundance of obligate subterranean species (Figure 2c) were best explained by the distance of the pitfall trap from the entrance; other variables had no significant effects and were dropped during model selection (Table 2). α-diversity (Figure 2b) and abundance (Figure 2d) of non-obligate species were best explained by the distance of the pitfall trap from the entrance in interaction with the sampling season. Specifically, we observed higher α-diversity and abundance at lower depth in summer, and at greater depth in winter. Moreover, abundance values were significantly lower in winter (Figure 2e, 2f). Finally, there was an effect of elevation on α-diversity and abundance of non-obligate species, with higher values at lower elevations (Table 2).

β-diversity

Results of β-diversity analysis are illustrated in Figure 3. In obligate subterranean species, the density of β_{Total} values across sites showed a bimodal concentration around 0 and 1, indicating the existence of two types of communities; β_{Total} values close to zero corresponded to the comparisons between pitfall traps with similar depth and environmental conditions, and values close to 1 to the comparisons between pitfall traps installed close to versus far from the entrance. This bimodal distribution was more pronounced in summer than in winter (Figure 3a). Nevertheless, $\beta_{Replacement}$ values were mostly concentrated towards zero (Figure 3b), indicating that community turnover in obligate-species was not due to a difference in species composition. There was a significant difference between $\beta_{Replacement}$ values for summer and winter ($W= 235950$, $p< 0.001$), whereas no significant differences between $\beta_{Richness}$ values in the two seasons were observed ($W = 9418.5$; $p= 0.22$; Figure 3c).

We observed pronounced seasonal variations in the distribution of non-obligate arthropod communities across sites (Figure 3d). In summer, there was a homogenization of values for non-obligate species, indicating a more even distribution of species among sites (Figure 3f, 3g). The richness component of β-diversity was found to fluctuate more across sites. Summer values were mostly concentrated either between 0.25 or between 0.75, whereas in winter the highest density of values was between 0.75 and 1. Observed differences between the two seasons were highly significant for $\beta_{Richness}$ ($W= 156080$; $p<0.001$) and approached significant for $\beta_{Replacement}$ ($W= 207350$, $p= 0.05$)

Discussion

Our expectation that community composition should be equal across the caves of the cave system of Comba dell'Infernotto was not entirely met. While a pool of nine obligate subterranean species was consistently found in most caves (Appendix S1), we observed significant turnovers in species composition between external and internal sectors of caves. When considering non-obligate subterranean species, there was also substantial turnover in richness and community composition, which was mostly attributable to seasonality.

The main driver of α-diversity and abundance was the distance of the pitfall trap from the entrance. Pitfalls at greater distance from the cave entrance yielded greater diversity and abundance of obligate subterranean species, independently from the sampling season. In our study site, a higher distance from the entrance (corresponding to greater values of subjacency) ensured the maintenance of more stable environmental conditions, including higher relative humidity and constant temperature (Figure 4). These are the optimal microclimatic conditions for the specialized subterranean fauna, and indeed subterranean habitats with grater environmental stability often support a more diverse and abundant subterranean arthropod community (Tobin *et al.*, 2013; Bento *et al.*, 2016; Mammola *et al.*, 2017).

In a few cases, obligate subterranean species also occurred in close proximity to the surface, although in low numbers. This was not an unexpected pattern, as it has been shown that obligate subterranean species within different cave systems may present a bimodal distribution, with peaks in richness and abundance in the nearby of the surface and deeper inside caves (Novak *et al.*, 2012; Kozel *et al.*, 2019). Yet, in the system of caves system of Comba dell'Infernotto, the predicted and observed abundance in the vicinity of the surface was close to zero (Figure 2d), which explains the non-significance of this pattern.

While obligate subterranean species were substantially unaffected by seasonal dynamics, the occurrence of non-obligate species varied significantly between winter and summer in relation to the distance of the sampling plot from the cave entrance. In winter, we mostly collected non-obligate species far from the cave entrance, where environmental conditions are relatively stable through the year and where temperatures never drop below the freezing point (Novak et al., 2014) (Figure 4). Winter abundance of accidental species was in general low, following the general reduction of activity in surface habitats in winter and the typical winter dormancy pattern in most arthropods. In winter, we collected the few non-obligate species in pitfall traps installed far from the entrance; these were mostly species overwintering in caves – a seasonal behaviour documented in several taxa (Chelini *et al.*, 2011; Lipovšek *et al.*, 2016, 2019; Balogová *et al.*, 2017; Mammola & Isaia, 2018).

On the other hand, in summer we observed a massive colonization of the shallow sectors of caves by external species. The importance of these species for the ecology of subterranean ecosystems is noteworthy, as they provide significant inputs of carbon into the system (Novak *et al.*, 2013). Concomitantly, there was also a migration of non-obligate species from deep toward shallow cave sectors. This pattern is often observed when studying spatial and temporal dynamics of temperate cave invertebrate communities (Tobin *et al.*, 2013; Lunghi *et al.*, 2017; Mammola & Isaia, 2018). In our case, the most abundant species involved in this migration was the predatory beetle *Sphodropsis ghiliani* (Schaum). In summer, this species seemingly moves into warmer, entrance sectors where the availability of prey is usually higher (e.g., Novak et al., 2013; Mammola & Isaia, 2018). The same migration pattern has been also observed for the same species in a cave of the North-Western Italian Alps (Mammola *et al.*, 2015).

We also found higher richness and abundance of non-obligate subterranean species at lower elevations (Table 3). In the study area, the elevation difference determines a change in the average temperature of about 1 °C between lower (1000 m) and higher (1200 m) caves, which may in turn drive the observed biological pattern. Alternatively, the explanation for this relationship may be linked to gravitation-assisted patterns in the distribution of species. Trontelj et al. (2019) documented how the distribution of animals within karst massif can be influenced by gravity. They argued that

297 "[…] *animals from a wider area of shallow subterranean spaces can be funneled towards deeper*
298 *parts of vertical caves by flowing water or gravity.*" This may explain why, including our case, caves
299 at lower elevations yielded a greater diversity and abundance of species.

300

301 **Conclusions**

302 Our results indicate that at a spatial scale of less than 1 km^2, there is a single pool of obligate species
303 that permanently occurs in the subterranean domain, and a pool of non-obligate species whose
304 presence in the subterranean domain is more influenced by seasonality. This finding corroborates the
305 idea that caves in close proximity are often interconnected by networks of subterranean habitat spaces
306 (Giachino & Vailati, 2010; Trontelj *et al.*, 2019; Mammola, 2020). Yet, the theoretical expectation of
307 no turnover in the community composition of these caves is not always met. Turnover is primarily
308 attributable to active migrations of fauna from deep to superficial habitats, and vice versa. From a
309 methodological perspective, our results imply that sampling a few caves across different seasons
310 should provide a general indication of the resident subterranean fauna of a certain area, having in
311 mind that a large depth is essential for sampling specialized subterranean taxa and that a greater
312 diversity is generally expected at lower elevations within a karst massif.

313

314 **Acknowledgements**

315 This work was funded by University of Turin and Compagnia di San Paolo (Grant Award:
316 CSTO162355). Special thank to Filippo Milano (University of Torino, Italy) for fieldwork assistance
317 and to Giulio Gardini (Genova, Italy) for the identification of pseudoscorpions. We are also grateful
318 to Dr. Dan Chamberlain for proof-reading our English.

319

320 **Supplementary material**

321 **Appendix S1**. List of species recorded in the system of caves of Maissa.

Literature cited

Balogová, M., Jelić, D., Kyselová, M. & Uhrin, M. (2017) Subterranean systems provide a suitable overwintering habitat for Salamandra salamandra. *International Journal of Speleology*, **46**, 321–329.

Bento, D. de M., Ferreira, R.L., Prous, X., Souza-Silva, M., Bellini, B.C. & Vasconcellos, A. (2016) Seasonal variations in cave invertebrate communities in the semiarid Caatinga, Brazil. *Journal of Cave and Karst Studies*, **78**, 61–71.

Cardoso, P., Mammola, S., Rigal, F. & Carvalho, J.C. (2020) BAT: Biodiversity Assessment Tools.

Cardoso, P., Rigal, F. & Carvalho, J.C. (2015) BAT – Biodiversity Assessment Tools, an R package for the measurement and estimation of alpha and beta taxon, phylogenetic and functional diversity. *Methods in Ecology and Evolution*, **6**, 232–236.

Cardoso, P., Rigal, F., Carvalho, J.C., Fortelius, M., Borges, P.A. V., Podani, J., *et al.* (2014) Partitioning taxon, phylogenetic and functional beta diversity into replacement and richness difference components. *Journal of Biogeography*, **41**, 749–761.

Carvalho, J.C., Cardoso, P. & Gomes, P. (2012) Determining the relative roles of species replacement and species richness differences in generating beta-diversity patterns. *Global Ecology and Biogeography*, **21**, 760–771.

Chelini, M.C., Willemart, R.H. & Gnaspini, P. (2011) Caves as a Winter Refuge by a Neotropical Harvestman (Arachnida, Opiliones). *Journal of Insect Behavior*, **24**, 393–398.

Chesta, M. & Elia, E. (2004) L'Infernotto e le grotte miniere della Maissa. *Mondo Ipogeo, Bollettino del Gruppo Speleologico Alpi Marittime*, **16**, 62–77.

Culver, D.C. (1970) Analysis of Simple Cave Communities I. Caves as Islands. *Evolution*, **24**, 463–474.

Culver, D.C. & Pipan, T. (2010) Superficial subterranean habitats – gateway to the subterranean realm? *Cave and Karst Science*, **35**.

Esposito, L.A., Bloom, T., Caicedo-Quiroga, L., Alicea-Serrano, A.M., Sánchez-Ruíz, J.A., May-Collado, L.J., *et al.* (2015) Islands within islands: Diversification of tailless whip spiders (Amblypygi, Phrynus) in Caribbean caves. *Molecular Phylogenetics and Evolution*, **93**, 107–117.

Fattorini, S., Borges, P.A.V., Fiasca, B. & Galassi, D.M.P. (2016) Trapped in the web of water: Groundwater-fed springs are island-like ecosystems for the meiofauna. *Ecology and Evolution*, **6**, 8389–8401.

Ficetola, G.F., Barzaghi, B., Melotto, A., Muraro, M., Lunghi, E., Canedoli, C., *et al.* (2018) N-mixture models reliably estimate the abundance of small vertebrates. *Scientific Reports*, **8**, 10357.

Ficetola, G.F., Canedoli, C. & Stoch, F. (2019) The Racovitzan impediment and the hidden biodiversity of unexplored environments. *Conservation Biology*, **33**, 214–216.

Gelman, A. & Hill, J. (2007) *Data analysis using regression and multilevel/hierarchical models*.

Cambridge University Press, Cambridge.

Giachino, P.M. & Vailati, D. (2010) *The subterranean environment. Hypogean life, concepts and collecting techniques.* WBA Handbo. Verona.

Howarth, F.G. (1983) Ecology of Cave Arthropods. *Annual Review of Entomology*, **28**, 365–389.

Itescu, Y. (2019) Are island-like systems biologically similar to islands? A review of the evidence. *Ecography*, **42**, 1298–1314.

Jarzyna, M.A. & Jetz, W. (2018) Taxonomic and functional diversity change is scale dependent. *Nature Communications*, **9**, 2565.

Jiménez-Valverde, A., Sendra, A., Garay, P. & Reboleira, A.S.P.S. (2017) Energy and speleogenesis: Key determinants of terrestrial species richness in caves. *Ecology and Evolution*, **7**, 10207–10215.

Kozel, P., Pipan, T., Mammola, S., Culver, D.C. & Novak, T. (2019) Distributional dynamics of a specialized subterranean community oppose the classical understanding of the preferred subterranean habitats. *Invertebrate Biology*, **138**, e12254.

Lipovšek, S., Leitinger, G., Janžekovič, F., Kozel, P., Dariš, B., Perc, M., *et al.* (2019) Towards understanding partial adaptation to the subterranean habitat in the European cave spider, Meta menardi: An ecocytological approach. *Scientific Reports*, **9**, 9121.

Lipovšek, S., Novak, T., Janžekovič, F., Weiland, N. & Leitinger, G. (2016) Malpighian tubule cells in overwintering cave crickets Troglophilus cavicola (Kollar, 1833) and T. neglectus Krauss, 1879 (Rhaphidophoridae, Ensifera). *PLoS ONE*, **11**, e0158598.

Lüdecke, D., Makowski, D. & Waggoner, P. (2020) performance: Assessment of Regression Models Performance.

Lunghi, E., Manenti, R. & Ficetola, G.F. (2017) Cave features, seasonality and subterranean distribution of non-obligate cave dwellers. *PeerJ*, **5**, e3169.

Mammola, S. (2019) Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. *Ecography*, **42**, 1331–1351.

Mammola, S. (2020) On deepest caves, extreme habitats, and ecological superlatives. *Trends in Ecology & Evolution*.

Mammola, S., Cardoso, P., Angyal, D., Balázs, G., Blick, T., Brustel, H., *et al.* (2019) Local- versus broad-scale environmental drivers of continental β-diversity patterns in subterranean spider communities across Europe. *Proceedings of the Royal Society B: Biological Sciences*, **286**, 20191579.

Mammola, S., Giachino, P.M., Piano, E., Jones, A., Barberis, M., Badino, G., *et al.* (2016) Ecology and sampling techniques of an understudied subterranean habitat: the Milieu Souterrain Superficiel (MSS). *The Science of Nature*.

Mammola, S. & Isaia, M. (2014) Niche differentiation in Meta bourneti and M. menard (Araneae, Tetragnathidae) with notes on the life history. *International Journal of Speleology*, **43**, 343–353.

Mammola, S. & Isaia, M. (2018) Day-night and seasonal variations of a subterranean invertebrate

community in the twilight zone. *Subterranean Biology*, **27**, 31–51.

Mammola, S., Piano, E., Giachino, P.M. & Isaia, M. (2015) Seasonal dynamics and micro-climatic preference of two alpine endemic hypogean beetles. *International Journal of Speleology*, **44**, 239–249.

Mammola, S., Piano, E., Giachino, P.M. & Isaia, M. (2017) An ecological survey of the invertebrate community at the epigean/hypogean interface. *Subterranean Biology*, **24**, 27–52.

Nagelkerke, N.J.D. (1991) A note on a general definition of the coefficient of determination. *Biometrika*, **78**, 691–692.

Niemiller, M.L. & Zigler, K.S. (2013) Patterns of cave biodiversity and endemism in the Appalachians and Interior Plateau of Tennessee, USA. *PLOS ONE*, **8**, e64177.

Novak, T., Janžekovič, F. & Lipovšek, S. (2013) Contribution of non-troglobiotic terrestrial invertebrates to carbon input in hypogean habitats. *Acta Carsologica*, **42**, 301–309.

Novak, T., Perc, M., Lipovšek, S. & Janžekovič, F. (2012) Duality of terrestrial subterranean fauna. *International Journal of Speleology*, **41**, 181–188.

Novak, T., Šajna, N., Antolinc, E., Lipovšek, S., Devetak, D. & Janžekovič, F. (2014) Cold tolerance in terrestrial invertebrates inhabiting subterranean habitats. *International Journal of Speleology*, **43**, 265–272.

Pipan, T. & Culver, D.C. (2017) The unity and diversity of the subterranean realm with respect to invertebrate body size. *Journal of Cave and Karst Studies*, **79**, 1–9.

R Core Team. (2018) R: A Language and Environment for Statistical Computing.

Růžička, V. (1982) Modifications to improve the efficiency of pitfall traps. *Newsletter of the British Arachnological Society*, **34**, 2–4.

Sket, B. (2008) Can we agree on an ecological classification of subterranean animals? *Journal of Natural History*, **42**, 1549–1563.

Snowman, C. V., Zigler, K.S. & Hedin, M. (2010) Caves as islands: mitochondrial phylogeography of the cave-obligate spider species Nesticus barri (Araneae: Nesticidae). *Journal of Arachnology*, **38**, 49–56.

Stoch, F. & Galassi, D.M.P. (2010) Stygobiotic crustacean species richness: a question of numbers, a matter of scale. *Hydrobiologia*, **653**, 217–234.

Tobin, B.W., Hutchins, B.T. & Schwartz, B.F. (2013) Spatial and temporal changes in invertebrate assemblage structure from the entrance to deep-cave zone of a temperate marble cave. *International Journal of Speleology*, **42**, 203–214.

Trajano, E. & Carvalho, M.R. de. (2017) Towards a biologically meaningful classification of subterranean organisms: A critical analysis of the schiner-racovitza system from a historical perspective, difficulties of its application and implications for conservation. *Subterranean Biology*, **22**, 1–26.

Trontelj, P., Borko, Š. & Delić, T. (2019) Testing the uniqueness of deep terrestrial life. *Scientific*

Reports, **9**, 15188.

Uéno, S.-I. (1987) The derivation of terrestrial cave animals. *Zoological Science*, **4**, 593–606.

Wynne, J.J., Howarth, F.G., Sommer, S. & Dickson, B.G. (2019) Fifty years of cave arthropod sampling: Techniques and best practices. *International Journal of Speleology*, **48**, 33–48.

Wynne, J.J., Sommer, S., Howarth, F.G., Dickson, B.G. & Voyles, K.D. (2018) Capturing arthropod diversity in complex cave systems. *Diversity and Distributions*, **24**, 1478–1491.

Zagmajster, M., Eme, D., Fišer, C., Galassi, D., Marmonier, P., Stoch, F., *et al.* (2014) Geographic variation in range size and beta diversity of groundwater crustaceans: Insights from habitats with low thermal seasonality. *Global Ecology and Biogeography*, **23**, 1135–1145.

Zuur, A.F. & Ieno, E.N. (2016) A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution*, **7**, 636–645.

Zuur, A.F., Ieno, E.N. & Elphick, C.S. (2009) A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, **1**, 3–14.

TABLES & FIGURES CAPTIONS

Table 1. Main features of the investigated caves.

Code (Fig.1)	Cave name	Cadaster code	Type	Elevation [m]	Pitfall distance from entrance [m]	Pitfall subjacency [m]	Total length [m]	Depth [m]	Entrance size [m^2]	Subjacency [m]	Longitude (°)	Latitude (°)
1	Maissa 1	1209 Pi	Natural	1186	16	16	37	–9	10	16	7.4050	44.2615
2	Topalinda – Maissa 2	1210 Pi	Natural	1179	36	30	334	–63	20	83	7.4052	44.2615
3	Maissa 16	1221 Pi	Natural	1169	4	7	12	6	4	3	7.4054	44.2615
4	Grotta dell'Ola – Maissa 23	1228 Pi	Natural	1105	4.5	2	6	–2	2.8	2	7.4060	44.2627
5	Grotta Diana – Maissa 22	1227 Pi	Natural	1048	19.1	14	39	–11	5	18	7.4061	44.2627
6	Small cave near Grotta Diana	n.c.	Natural	1111	3.1	8	4	0	2.7	8	7.4065	44.2622
7	Maissa 21	1226 Pi	Natural	1145	3	4	6	–2	2	4	7.4168	44.2614
8	Maissa 9	1217 Pi	Natural	1085	10.3	7	11	–1	2.5	7	7.4084	44.2617
9	Maissa 8	1216 Pi	Natural	1077	6.4	7	6	–2	1.2	9	7.4084	44.2618
10	Maissa 7	1215 Pi	Natural	1071	35	13	29	–11	1.3	13	7.4086	44.2618
11	Small cave near Baron Litron	n.c.	Natural	1063	5.5	3	10	–1	12	5	7.4093	44.2616
12	Barôn Litrôn – Maissa 6	1214 Pi	Natural/Mine	1063	16	38	861	–59	10	75	7.4093	44.2616
13	Sweet Inny – Maissa 10	1218 Pi	Natural	1045	14.3	13	116	–30	35	35	7.4098	44.2615
14	Small cave near Maissa 12	n.c.	Natural	1027	12	6	12	–7	1.5	6	7.4099	44.2613
15	Small mine shaft near Maissa 11	n.c.	Mine	1038	2.5	1	4	–1	0.5	1	7.4102	44.2617
16	Small cave near Maissa 11	n.c.	Natural	1051	3.5	2	5	0	26	1	7.4104	44.2613
17	Maissa 12	1219 Pi	Natural	1090	13.3	12	92	–20	2.6	14	7.4106	44.2613
18	Small cave near Maissa 10	n.c.	Natural	1037	3.5	2	8	3	3	2	7.4105	44.2617
19	Maissa 11	n.c.	Natural	1054	6.8	7	37	–20	0.8	22	7.4104	44.2613
20	Maissa 13	n.c.	Natural	1023	22	5	20	–4	3	5	7.4113	44.2619
21	Grotta dei Morti	1054 Pi	Natural	1023	19	15	84	–16	10	15	7.4131	44.2611
22	Mineshaft near Grotta Infernotto Superiore	n.c.	Mine	1041	5.5	4	6	–1	4.2	4	7.4113	22.2605
23	Grotta Infernotto Superiore	1055 Pi	Natural	1041	6.1	4	19	–1	2.5	12	7.4130	44.2609
24	Maissa 31	n.c.	Natural	1181	3.4	3	7	–2	3	3	7.4148	44.2580
25	Maissa 32	n.c.	Natural	1177	8.2	3	10	4	2	3	7.4148	44.2580
26	Natural cave “Salamandre”	n.c.	Natural	1100	3.6	4	7	–2	2.5	4	7.4144	44.2580
27	Natural cave “Salamandre Superiore”	n.c.	Natural	1110	2	5	10	2	1.5	5	7.4144	44.2580

n.c. = mines or caves not included in the speleological cadaster; subjacency = vertical distance from the surface; Mammola et al. 2017)

Table 2. Results of model selection. For each dependent variable, models are listed from the least to the most supported. AIC = Aikaike Information Criterion.

Dependent variable	Model structure	AIC
Richness of obligate subterranean species (α)	Pitfall depth + Season + Elevation + Entrance size	129.24
	Pitfall depth + Elevation + Entrance size	127.46
	Pitfall depth + Elevation	126.17
	Pitfall depth	125.18
Abundance of obligate subterranean species	Pitfall depth + Season + Elevation + Entrance size	264.60
	Pitfall depth + Elevation + Entrance size	262.62
	Pitfall depth + Elevation	261.17
	Pitfall depth	259.75
Richness of non-obligate species (α)	Pitfall depth * Season + Elevation + Entrance size	227.16
	Pitfall depth * Season + Elevation	225.15
Abundance of non-obligate species	Pitfall depth * Season + Elevation + Entrance size	437.80
	Pitfall depth * Season + Elevation	435.82

Table 3. Estimated regression parameters in the Minimum Adequate Models. R^2 = Nagelkerke's pseudo-R^2 (Nagelkerke, 1991).

Dependent variable	**Variable**	**Estimated β ± S.E.**	***p*-value**	**R^2**
Richness of Troglobionts (α)	Intercept	–1.98 ± 0.50	-	0.50
	Distance from the entrance	0.85 ± 0.18	<0.001	
Abundance of Troglobionts	Intercept	–3.10 ± 0.69	-	0.70
	Distance from the entrance	1.98 ± 0.28	<0.001	
Richness of Troglophiles (α)	Intercept	8.66 ± 1.54	-	0.87
	Distance from the entrance	–0.44 ± 0.12	<0.001	
	Season (Winter)	–3.12 ± 0.51	<0.001	
	Distance from the entrance * Season	0.97 ± 0.21	<0.001	
	Elevation	–0.01 ± 0.01	<0.001	
Abundance of Troglophiles	Intercept	11.93 ± 2.82	-	0.91
	Pitfall depth	–0.38 ± 0.24	0.110	
	Season (Winter)	–6.34 ± 0.88	<0.001	
	Distance from the entrance * Season	1.54 ± 0.34	<0.001	
	Elevation	–0.01 ± 0.01	0.015	

Figure 1. Map of the study area. Dots show the distribution of the sampled caves on the left slope of the Comba dell'Infernotto (municipality of Valdieri, Province of Cuneo, Italy). Photographs illustrate the morphology of some examples of cave entrances.

Figure 2. Drivers of richness and abundance of species in the system of caves of Comba dell'Infernotto. Predicted relationships between the richness and abundance of obligate (a, b) and non-obligate (c, d) species and the depth at which the pitfall trap was placed in each cave, based on the results of regression analysis. Note that for non-obligate species, there was a significant interaction between the depth of the pitfall trap and the sampling season; therefore, the predicted relationship is shown separately for each season. In all predictions of non-obligate species, elevation is set at the mean values. Black lines are predicted values, while grey surfaces are 95% confidence intervals.

Figure 3. Community turnover in the system of caves of Comba dell'Infernotto. Density of β-diversity values for obligate (a, b, c) and non-obligate (d, e, f) terrestrial arthropod communities in winter and summer samples. Total β-diversity is split in two components: $\beta_{richness}$ is the turnover in community composition explained by species loss/gain alone, and $\beta_{replacement}$ is turnover in community composition explained by replacement of species alone.

Figure 4. Example of cave microclimate in the system of caves of Comba dell'Infernotto. Daily temperature was registered by two dataloggers, one placed at a depth of 36 m in Maissa 2 and one at 4.5 m in Maissa 23.